UNIVERSITI PUTRA MALAYSIA

MANGANESE ACCUMULATION AND TOXICITY, AND SUPEROXIDE DISMUTASE ACTIVITY IN VEGETABLE SOYBEAN (GLYCINE MAX (L.) MERR.)

ROSMAIDAR DJAFRUDDIN

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By

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DOCTOR OF PHILOSOPHY
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ROSMAIDAR DJAFRUDDIN

Thesis Submitted to the Graduate School, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

September 2001

"Especially to my son Romiandi

Life is so simple my dear son
Initiation,
Differentiation and finally
Termination

Therefore, my son
Remember your God (ALLAH s.w t) always,
Perform the "shalat" regularly

That is key of life
Key for success

PS to my lovely brothers and sisters"

Memorial to my father who suffering against cancer
My mother who pain in Xeroxes
Never release losing you

Abstract of thesis submitted to senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

MANGANESE ACCUMULATION AND TOXICITY, AND SUPEROXIDE DISMUTASE ACTIVITY IN VEGETABLE SOYBEAN (*GLYCINE MAX* (L.) MERR.)

By

ROSMAIDAR DJAFRUDDIN

September 2001

Chairman : Dr. Anuar Abd Rahim

Faculty : Agriculture

Manganese toxicity is one of the growth limiting factors under acidic conditions. Some plants accumulate Mn more in the top than in the root, while others accumulate more in the root than in the top. Vegetable soybean is an indicator plant that can be used to determine the mechanism of toxicity. Part of the plant tissue that accumulates the highest Mn needs to be extracted so as to determine the activity of superoxide dismutase (SOD). In view of this, a series of experiments was conducted to determine the accumulation of Mn in the tissues (leaf, stem, root and pod) of vegetable soybean, toxic level of Mn in solution culture, time-dependent of Mn accumulation in the tissue and the effect

of Mn concentration on superoxide dismutase activity under different time of plant growth.

Five experiments were conducted in glass house and laboratories at UPM. Experiment 1 was a sand culture experiment to determine the distribution of Mn accumulation in the plant tissues. Experiment 2 and 3 were solution culture experiments to observe toxic level of Mn. Experiment 4 was used to determine time-dependent of Mn accumulation, while experiment 5 was used to study the effect of Mn and time on superoxide dismutase activity.

The results showed that the highest accumulation of Mn in vegetable soybean was in the leaves. The Mn concentration corresponding to 20 % reduction in dry weight was 20 μM. Plant accumulated Mn more during vegetative stage compared to generative stage. Increasing the time of growth decreased the accumulation of Mn in the 5 and 25 μM of Mn treatments. Degeneration of plant cell in toxic condition (25 μM) affected the growth of plant and inhibited nutrient movement from the root to the shoot. Enzyme extraction showed that the activity of superoxide dismutase increased up to 25.36 μmol mL^{-1} of crude enzyme $minute^{-1}$ at level 15 μM after 20 days of plant age in solution culture.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

AKUMULASI MANGAN DAN KETOKSIKAN, DAN AKTIVITI SUPEROKSIDA DISMUTASI PADA KACANG SOYA SAYURAN (*GLYCINE MAX* (L.) MERR.)

Oleh

ROSMAIDAR DJAFRUDDIN

September 2001

Pengerusi : Dr. Anuar Abd Rahim

Fakulti : Pertanian

Ketoksikan mangan (Mn) adalah salah satu faktor penghalang tumbesaran dalam keadaan berasid. Beberapa tanaman mengumpul Mn pada bahagian atas lebih banyak berbanding akar, sementara yang lain mengumpul pada akar lebih banyak berbanding bahagian atas. Kacang soya sayuran adalah tanaman penunjuk digunakan untuk menguji mekanisme ketoksikan Mn. Bahagian tisu yang boleh mengumpul Mn dengan banyak diperlukan untuk mengekstrak bagi menentukan aktiviti superoksida dismutasi (SOD). Berdasarkan masalah ini, beberapa kajian dijalankan untuk menguji pengumpulan Mn pada setiap bahagian tisu kacang soya sayuran (daun, batang, akar dan buah), paras toksik Mn dalam larutan nutrien, pergantungan

masa pengumpulan Mn di dalam tisu dan pengaruh kepekatan Mn terhadap aktiviti superoksida dismutasi dalam tempoh masa tumbesaran.

Lima kajian telah dijalankan di rumah kaca dan makmal UPM. Kajian 1 adalah untuk menentukan penyebaran pengumpulan Mn di dalam tisu tanaman menggunakan kultura pasir. Kajian 2 dan 3 adalah kajian di dalam larutan nutrien untuk menentukan paras toksik Mn. Kajian 4 dijalankan untuk menentukan pergantungan masa pengumpulan Mn, manakala kajian 5 untuk mengkaji pengaruh kepekatan Mn dan masa terhadap aktiviti superoksida dismutasi (SOD).

Keputusan kajian menunjukkan pengumpulan Mn tertinggi adalah pada daun kacang soya berbanding batang, akar dan buah. Paras Mn yang bersamaan dengan 20 % penurunan berat kering adalah 20 μM. Kacang soya sayuran mengumpul Mn lebih tinggi pada masa vegetatif berbanding reproduktif. Penambahan masa tumbesaran mengurangkan pengumpulan Mn pada 5 dan 25 μM Mn. Kemerosotan pertumbuhan sel pada tanaman dalam keadaan toksik Mn (25 μM) telah mempengaruhi tumbesaran tanaman dan mengganggu pergerakan nutrien dari akar ke pucuk. Pengekstratan enzim menunjukkan bahawa aktiviti superoksida dismutasi meningkat sehingga 25.36 μmol mL^{-1} enzim mentah $minit^{-1}$ pada 15 μM selepas 20 hari di dalam larutan nutrien.

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I certify that an Examination Committee met on 24th September 2001 to conduct the final examination of Rosmaidar Djafruddin on her Doctor of Philosophy thesis entitled "Manganese Accumulation and Toxicity, and Superoxide Dismutase Activity in Vegetable Soybean (*Glycine max* (L.) Merr.)" in accordance with Universiti Pertanian Malaysia (Higher degree) Act 1980 and Universiti Pertanian Malaysia (Higher degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

Rosmaidar Djafruddin

Date : 15-2-2002

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CHAPTER ONE

INTRODUCTION

Soybean (*Glycine max* (L.) Merr.) is one of the important crops that has a very significant, diverse and the variety that can be used is wide range, in different food items. The United States (US) competes with China in the production of soybean for the purposes of manufacturing various soyfoods. In China soybean is used as vegetable, an important part of daily diet (Plucknett and Halsey, 1981), while in US soybean is famous as vegetable legume an important species as food source for human and animal (Vincent, 1997).

Special varieties of soybeans can be used as 'vegetable' if they are picked at the right stage. Vegetable soybean contains about 16 % protein and is rich in minerals, especially Ca, and vitamins A and B (Mookherji and Floyd, 1991). Vegetable soybean is very famous in Japan as table food. Hence, production of healthy soybean crop becomes very important all around the world. Soybean and other similar crops like potato, beetroot and others are adversely affected by the presence of naturally occurring Mn absorbed directly from the soil.